Detection of minimal residual disease in adult acute myeloid leukemia via CD25

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Research article

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Abstract

Background We detected the expression of CD25 in patients with acute myeloid leukemia (AML) to value whether CD25 could be a promising marker for minimal residual disease (MRD). Methods Two hundred and twenty bone marrow (BM) specimens from 98 adult patients with AML after chemotherapy were detected using flow cytometry. The expression of CD25 was compared between MRD positive and negative subgroups. Results About 38% of patients with MRD were positive for CD25. The mean percentage of CD25-positive cell subpopulation was 58.68% relative to the whole MRD cluster (0.05%-100%). The mean fluorescence index ratio (MFIR) of CD25 in these cell subpopulations was approximately13-fold greater than that in normal myeloblasts. The detection sensitivity of CD25 was as high as 10 -4 . CD25 was also expressed on non-leukemic stem cells that were positive for CD34 and CD38. Conclusion CD25, as assessed by flow cytometry, is a promising marker for MRD in patients with AML.

Background

In acute myeloid leukemia (AML), the presence of minimal residual disease (MRD), in which the number of leukemic cells is lower than the detection threshold of morphology, is an independent prognostic parameter of relapse and survival [1-6]. Flow cytometry (FC), molecular techniques, and cytogenetic analysis are three prominent technologies for detecting MRD[1,4,7-10]. Generally, there are two approaches for MRD detection by FC: detection of cells with leukemia-associated immunophenotypes (LAIPs), which differ from normal hematopoietic cells [11-13]; detection of different from normal (DfN) patterns [10]. These approaches can also be combined as a “LAIP-based DfN approach” [14]. With the mounting number of LAIP [1,3] and the application of multiparameter FC, the detection sensitivities of FC can be as high as 1:103 to 1:106 in BM samples [10, 14, 15]. All these methods are based on antigens with abnormal expression. To reduce the false detection of MRD-negative ratios and expand the application scope, novel MRD markers need to be developed.

CD25 (interleukin-2 receptor) is expressed in 10%-20% of patients with AML [15,16]. This marker is correlated with FLT3-ITD, DNMT3A, and NPM1 mutations [16,17]. It has also been proven to be an independent, unfavorable prognostic parameter in AML [16-18]. More recently, CD25 has been detected in leukemic stem cells (LSCs) in patients with myelodysplastic syndrome [19]. Here, we used CD25 as a novel marker to detect MRD in patients with AML.

Methods

Patients

The BM samples were obtained with informed consent. The study was approved by the Ethics Committee of the Peking University International Hospital and was in compliance with the guidelines of the Helsinki Declaration of 2008. A total of 220 longitudinal BM specimens from 98 adult AML patients (55 males

and 43 females) were collected after chemotherapy from Peking University International Hospital between June 2017 and June 2019. Among the samples, 165 BM samples from 43 patients were collected at different time points after chemotherapy.

Flow cytometry

Fluorescent mouse anti-human monoclonal antibodies (mAbs) against the following markers were used: CD2, CD5, CD7, CD11b, CD13, CD14, CD15, CD19, CD25, CD33, CD34, CD36, CD38, CD41, CD45, CD56, CD61, CD64, CD71, CD117, CD123, CD235a, and HLA-DR (from BD Biosciences and Beckman Coulter). The MRD detection panels are listed in Additional file 1.

BM samples were collected in heparin anticoagulant tubes. A total of 5×105 white blood cells/tube were stained and detected within 12 hours after procurement. Data were acquired and analyzed using a BD FACSCalibur flow cytometer and Cell Quest software (from BD Biosciences).

MRD as determined by FC was defined as reported [14]. CD25 expression was calculated as the MFIR as described previously [20]. In MRD-negative BM samples, normal myeloblasts are positive for CD34, CD117, and CD45. Based on the expression profile of CD25, the MRD-positive BM samples were separated into two subgroups. If there was a CD25-positive subpopulation, the MFIR of CD25 applied only to this subpopulation instead of to the whole MRD cluster. In addition, the positive percentage of cells positive for CD25 in the MRD cluster was recorded. If the whole MRD cluster was positive for CD25, then the MFIR of CD25 applied to the whole cellular cluster.

PCR assay

All BM samples were analyzed by PCR. Five-milliliter BM samples were collected in in EDTA anticoagulant tubes. The PCR assay was performed based on previous reports [4,10,14] Fusion genes (PML-RARA, CBFB-MYH11, RUNX1-RUNX1T1, BCR-ABL1, MLLT3-KMT2A, and DEK-NUP214) and mutant genes (NPM1, CEBPA, RUNX1, FLT3, TP53, KIT, and ASXL1) were detected before treatment [8,21]. Samples from patients with mutant NPM1, PML-RARA, CBFB-MYH11, and RUNX1-RUNX1T1 mutations were subjected to PCR for MRD detection. MRD was defined based on previous reports [1,14]

Statistics

The 2-tailed Student's t-test was used to evaluate the difference in CD25 expression between the two different subgroups. A Receiver Operating Characteristic (ROC) Curve was used to set the CD25 MFIR cut-off value. All the statistical analyses were performed by using the GraphPad Prism 6 (GraphPad Software, San Diego, CA). The MFIR values are presented as the means ± standard errors of the mean (SEM).

Results

The expression of CD25

Firstly, we set up the CD25 MFIR cut-off value (5.46) by using the ROC curve. [Additional file 2] Of the MRD-positive AML patients, 38.27% (31/81) were positive for CD25. Of the 75 specimens positive for CD25, approximately 58.68% (0.05% to 100%) of the MRD cells were positive for CD25. Compared to the MRD-negative samples (1.73 ± 0.09), the MRD-positive samples had a significantly increased MFIR (23.22 ± 1.45, $p<0.05$). [Table 1, Figures1, 2, and Additional file 3] The sensitivity of CD25 for detecting MRD was 5/10,000.[Figure 3]

The stability of CD25 in MRD-positive AML

The stability of CD25 was dynamically monitored in 127 BM specimens from 40 MRD-positive patients. Among these specimens, the CD25 MFIR of 7 MRD-positive samples varied between normal and abnormal ranges [Figure 2 and Additional file 4]. The CD25 MFIR of the other patients remained stable in either the abnormal range or the normal range.

Clinical characteristics

AML patients were stratified into three risk categories based on genetic information before treatment[1,8,21]. Of the CD25-positive patients with MRD, 65% and 35% were in the intermediate and poor risk subgroups, respectively. Of the CD25-negative patients with MRD, 24%, 46% and 30% cases were in the favorable, intermediate and poor risk subgroups, respectively. Clinical information for all patients is listed in Table 1.

Other characteristics of CD25-positive cases with MRD

Of 31 MRD-positive AML patients expressing CD25, 6/31 cases were positive for concurrent FLT3-ITD and NPM1 mutations, another 6/31 exhibited FLT3-ITD and 3/31 exhibited NPM1 mutations. Additional genetic abnormalities of CD25-positive and CD25-negative patients with MRD are shown in Table 2.

We also observed the expressions of CD38 in 10 patients with MRD that were positive for CD25. Five patients were concurrently positive for CD34 and CD38, while three cases were positive for CD34 and negative for CD38, which is the immunophenotype of LSCs [22, 23]. One patient was negative for CD34 and positive for CD38.

Discussion

Previous reports on CD25 expression in AML have been focused on its prognostic value. In this research, we identify the value of CD25 as a novel MRD marker in AML.

We initially observed that the expression of CD25 in normal myeloblasts from MRD-negative cases was quite low. This finding suggested that CD25 is a potential marker for MRD in AML. Then, we set up the upper cut-off value for the CD25 MFIR by using a ROC curve and separated the MRD-positive AML samples into two subgroups. Approximately 38% of the MRD-positive AML patients were positive for CD25. Based on other reports, CD25 is expressed in 10%-20% of AML patients [15,16], and the numbers of

cases are much larger in these studies. Therefore, the bias toward the higher positive percentage of CD25 in our study may be caused by the small sample size. After comparing the CD25 MFIR between CD25-positive patients with MRD and those without MRD, we found that the difference was obvious. This result shows that high expression of CD25 is a reliable marker for MRD in AML.

We also compared the stability of CD25 in 127 BM specimens from 40 MRD-positive patients. The stability of CD25 varied between the normal and abnormal ranges in approximately 7 patients. This pattern means that the expression of CD25 could change at different time points. Therefore, it would be better to detect CD25 whenever MRD monitoring is performed.

In addition, we tried to determine the characteristics of CD25-positive patients with MRD. Regarding the genetic abnormalities, we obtained a similar conclusion as other reports [17]. FLT3-ITD and NPM1 mutations were the most common genetic aberrancies in AML patients positive for CD25. CD25 is highly expressed in LSCs [24] In addition, CD25-positive AML blasts express an LSC-like gene profile [17]. Generally, the combination of CD34+ and CD38- is the recognized immunophenotype of LSCs in AML [22,23]. However, based on our result, not all CD25 positive MRD clusters are negative for CD38. This finding might indicate that CD25 can be expressed in AML cells other than LSCs. However, in this study, we only used four-parameter flow cytometry.

Our group is not the first to use CD25 as an MRD detection marker in AML. A Japanese group detected the expression of CD25 in AML patients before allogeneic hematopoietic stem cell transplantation and found out that AML patients with CD25-positive MRD at the time of transplantation had poor prognoses [25].

To our knowledge, our group is the first to describe the application of CD25 for MRD detection in detail. In the future, we will include more cases and use multiple-parameter flow cytometry.

Conclusion

CD25 is a promising marker for MRD in patients with AML.

List Of Abbreviations

FC, flow cytometry

BM, bone marrow

AML, acute myeloid leukemia

MRD, minimal residual disease

MFIR, mean fluorescence index ratio

LAIPs, leukemia-associated immunophenotypes

DfN, different from normal

LSCs, leukemic stem cells

mAbs, monoclonal antibodies

ROC, Receiver Operating Characteristic

SEM, standard errors of the mean

Declarations

Ethics approval and consent to participate

The study has been approved by the Ethical by the Ethical Committee of the Peking University International Hospital (C2017-005) and has been performed in accordance with the ethical standards as described in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All patients were provided with informed written consent regarding the data collection and publication.

Consent to publish

We obtained written permission from all patients in the study to publish the manuscript reporting individual patient data.

Availability of data and materials

All data are presented in the manuscript and supplementary data.

Competing interests

None of the authors have any competing interests in regard to the manuscript.

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Authors' contributions

WW planned the entire research study, performed the flow cytometry experiments, and prepared the manuscript. YL performed the flow cytometry experiments and statistic analysis. LM collected patients information, and performed the statistic analysis. WQH collected patient samples, analyzed and interpreted the results. BJ supervised the entire study and revised the manuscript. All authors have read and approved the final manuscript.

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Tables

Table 1. Clinical information and laboratory results of AML patients after treatment

	CD25+MRD positive	CD25-MRD positive	MRD negative
Patient number [a]	31	50	36
BM number	75	87	58
Age	54 (19-77)	45 (18-81)	48 (18-82)
Sex (male/female)	17/14	29/21	22/14
Risk stratification of genetics			
Favorable	0	12	Not recorded
Intermediate	20	23	Not recorded
Adverse	11	15	Not recorded
MRD blasts/ BM nucleated cells (%) (mean ± SEM)	25.65 ± 3.35	14.83 ± 2.21	None
CD25 MFIR (mean ± SEM)	23.22 ± 1.45 [b]	1.59 ± 0.07 [c]	1.73 ± 0.09 [d]

AML: acute myeloid leukemia

MRD: minimal residual disease

BM: bone marrow

a: There were 98 patients in total. Among the patients, 43 were assessed at multiple time points. Some of these samples may be distributed in different subgroups.

b: The MFIR of CD25 in CD25 positive AML cells

c: The MFIR of CD25 in all AML cells

d: The MFIR of CD25 in normal myeloblasts

Table 2. Genetic information of MRD-positive AML patients

Genetic abnormalities	CD25+ MRD-positive	CD25- MRD-positive
PML-RARa	0	1
RUNX1-RUNX1T1	1	6
BCR-ABL1	2	0
MLLT3-KMT2A	3	6
NPM1 mutation[a]	9	9
Biallelic mutated *CEBPA*	0	2
RUNX1 mutation	1	6
FLT3-ITD	12	6
TP53 mutation	0	2
KIT mutation	1	1
ASXL1 mutation	1	3

MRD: minimal residual disease

a: Six patients in the CD25-positive subgroup and 3 cases in the CD25-negative subgroup had concurrent *NPM1* mutation and *FLT3-ITD*.

In addition, other genetic abnormalities were observed in some patients.

Additional File Legends

Additional file 1. The minimal residual disease detection panels in acute myeloid leukemia.

Additional file 2. Receiver Operating Characteristic (ROC) Curve of CD25 MFIR.

Cut off< 5.457. Sensitivity is 100% (93.84% to 100%); specificity is 46.3% (38.44% to 54.29%); area is 0.6887 (p<0.001).

Additional file 3. Laboratory data of CD25 positive and MRD positive AML patients

Additional file 4. The variability of CD25 MFIR in MRD positive AML patients

Figures

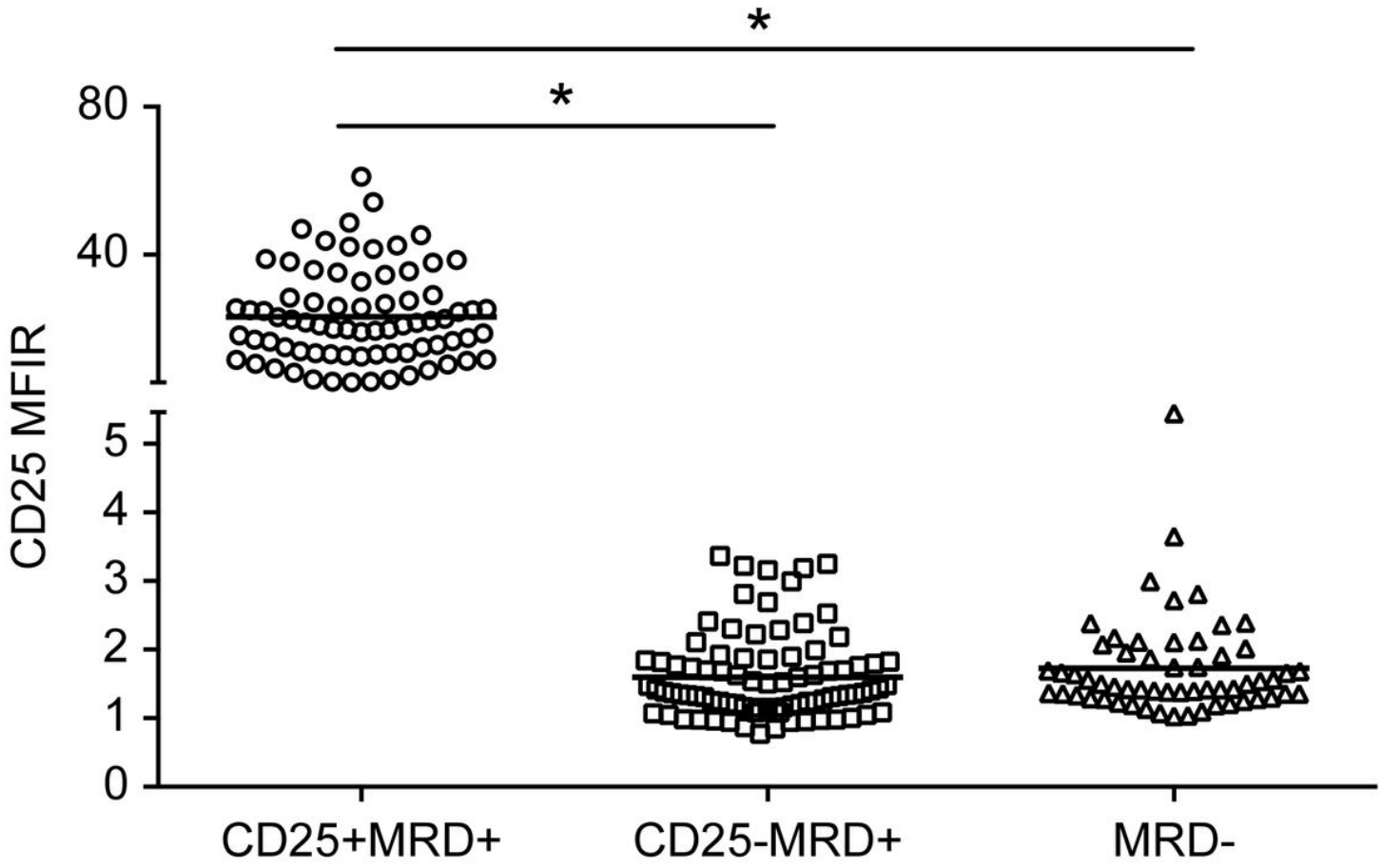

Figure 1

Expression of CD25 in AML patients after treatment. The results of flow cytometry are shown. Seventy-five specimens from MRD-positive patients were positive for CD25. The 2-tailed Student's t-test was used. The horizontal bars indicate the mean values. The star indicates a p-value lower than 0.05. MRD: minimal residual disease; MFIR: mean fluorescence intensity ratio; AML: acute myeloid leukemia.

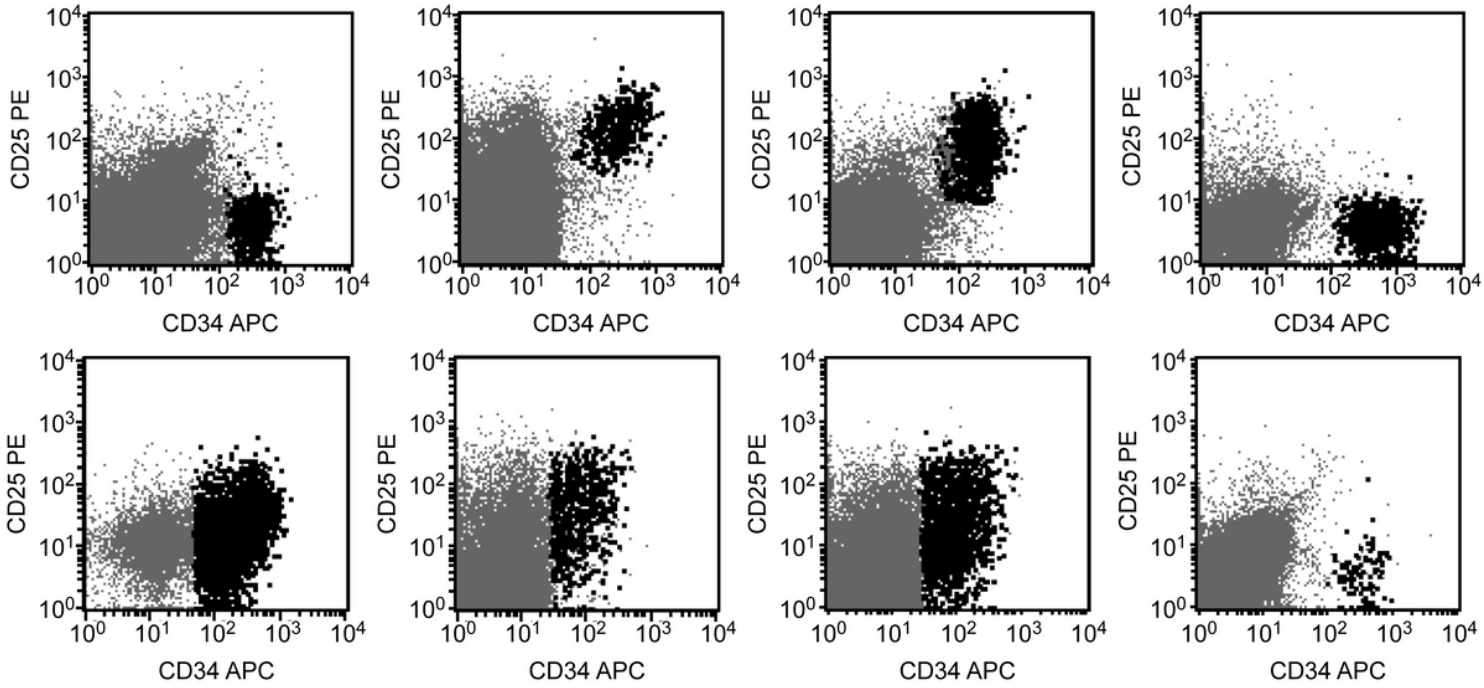

Figure 2

The expression profile of CD25 in blasts. From the first to the fourth figure shows the expression of CD25 in blasts. The first figure shows negative expression of CD25 in normal blasts from an AML patient without MRD. The second figure shows the positive expression of CD25 in the whole MRD cluster from an MRD-positive patient. The third figure shows positive expression of CD25 in a part of the MRD cluster from an MRD-positive patient. The fourth figure shows negative CD25 expression in the MRD cluster from an MRD-positive patient. From the fifth to the eighth figure shows the dynamic variation in CD25 expression in an MRD-positive patient. The expression of CD25 was partly positive in MRD clusters at the first, second, and third time points after chemotherapy. The expression of CD25 was negative in MRD clusters at the fourth time point after chemotherapy. MRD: minimal residual disease; AML: acute myeloid leukemia.

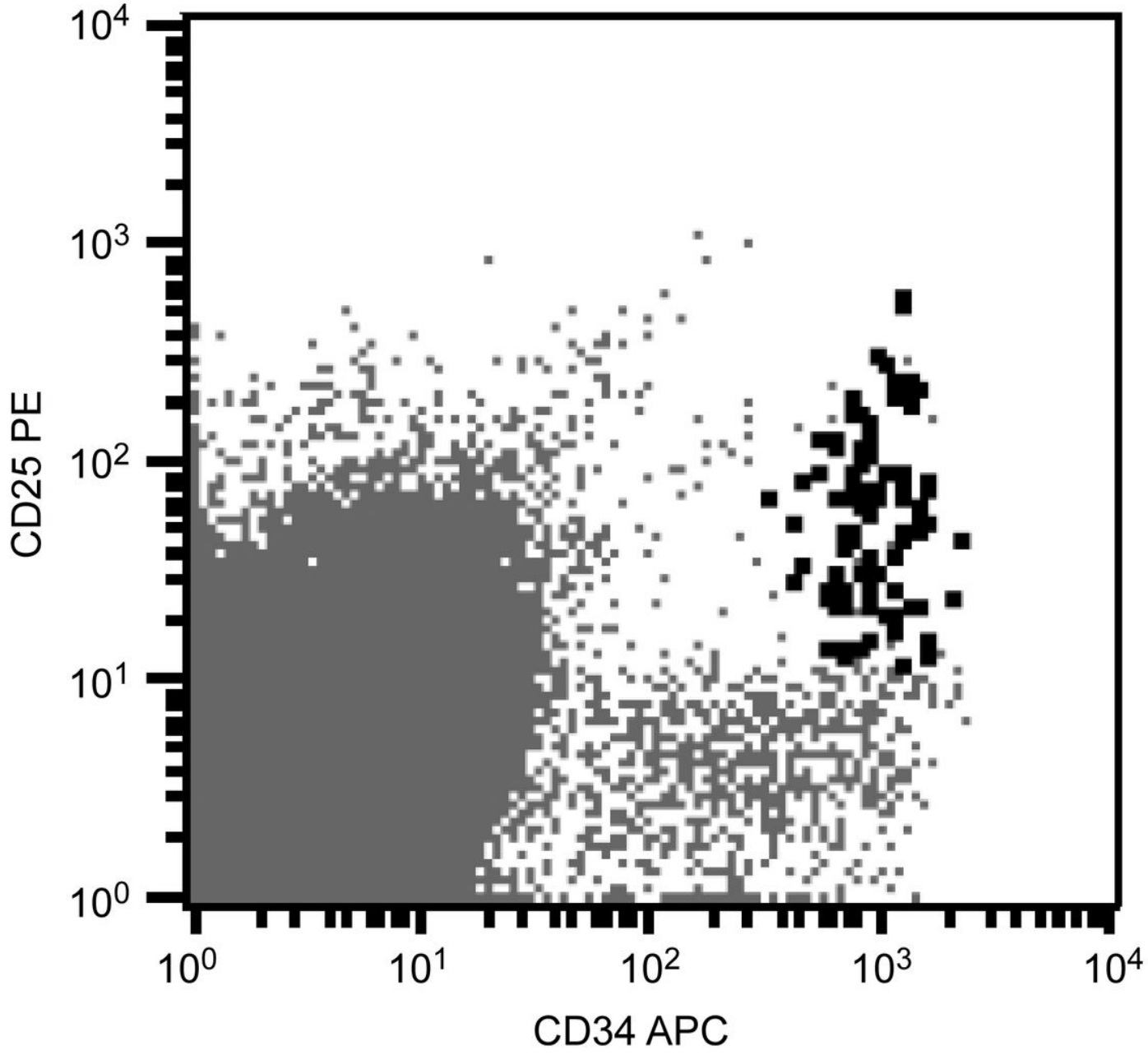

Figure 3

The sensitivity of CD25 in MRD detection. The detection sensitivity of CD25 positivity in the MRD cluster was 5/10,000 relative to all bone marrow nucleated cells. MRD: minimal residual disease.

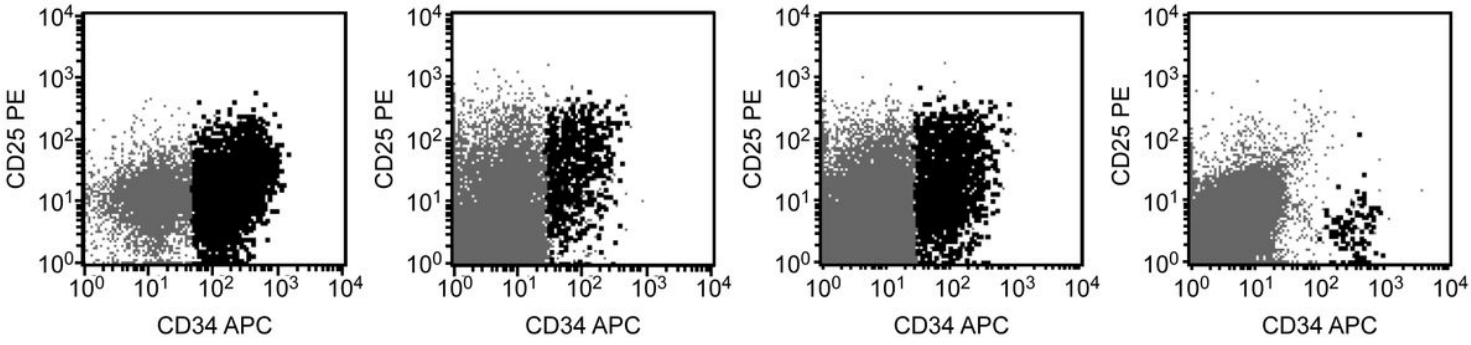

Figure 4

The stability of CD25 in the MRD cluster. The figure shows the dynamic variation in CD25 expression in an MRD-positive patient. The expression of CD25 was partly positive in MRD clusters at the first, second, and third time points after chemotherapy. The expression of CD25 was negative in MRD clusters at the fourth time point after chemotherapy.

Supplementary Files

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